Environmental Health Perspectives
Vol. 69, pp. 183-202, 1986

Toxicology of Haloacetonitriles

by Johnnie R. Hayes,*† Lyman W. Condie, Jr.,‡ and Joseph F. Borzelleca*

Haloacetonitriles are by-products of water chlorination and may form *in vivo* from the reaction of residual chlorine with endogenous compounds such as amino acids. Dibromoacetonitrile (DBAN) was negative in selected mutagenic assays; dichloroacetonitrile (DCAN) was mutagenic in *S. typhimurium*, but not in *S. cerevisiae*. Both DBAN and DCAN may be carcinogenic. There is a paucity of basic toxicological data for these compounds. The studies described were conducted to determine the acute, subacute, and subchronic toxicity of DBAN and DCAN. The acute oral LD_{50} values (mg/kg) in mice and rats are: DBAN, mice: 289 (M), 303 (F); DBAN, rats: 245 (M), 361 (F); DCAN, mice: 270 (M), 279 (F); DCAN, rats: 339 (M), 330 (F). Death was preceded by slowed respiration, depressed activity, prostration, and coma. There were no apparent compound-related gross pathological effects.

DBAN (in corn oil) was administered by gavage to male and female CD rats for 14 or 90 days at levels of 23, 45, 90, and 180 mg/kg/day or 6, 23, and 45 mg/kg/day, respectively. Mortality was 100% at 180 mg/kg and 40% (M) and 20% (F) at 90 mg/kg/day. Compound-related mortality was 10% (M) and 5% (F) at 45 mg/kg and 0% (M) and 10% (F) at 23 mg/kg during the 90-day study. No consistent, significant, adverse compound-related effects on any of the parameters evaluated were evident. Possible target organs might be spleen, thymus, and liver. The no-observed adverse-effect level (NOAEL) for 14 days was 45 mg/kg/day and for 90 days was 23 mg/kg/day.

DCAN (in corn oil) was administered by gavage to male and female CD rats for 14 or 90 days at levels of 12, 23, 45, and 90 mg/kg/day or 8, 33, and 65 mg/kg/day, respectively. There were no deaths during the 14-day study. Compound-related mortality was 50% (M) and 25% (F) at 65 mg/kg, 10% (M) and 5% (F) at 33 mg/kg, and 5% (M) and 0% (F) at 8 mg/kg during the 90-day study. Body weights were significantly lower at 90 and 65 mg/kg/day; weight and ratios of spleen and gonads and cholesterol levels were significantly lower at 90 mg/kg/day. No consistent, significant adverse compound-related effects on any of the parameters evaluated were evident. The NOAEL for 14 days was 45 mg/kg/day and for 90 days was 8 mg/kg/day.

Introduction

By-products of water chlorination include trihalomethanes (THMs) (*1–4*) and dihaloacetonitriles (DHAN) (*5–7*). For example, dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), and bromochloroacetonitrile (BCAN) were detected in chlorinated groundwater and surface water supplies of South Florida but were not found in finished waters with pH 9 or greater (*7*). Probable precursors of these DHANs include amino acids and humic acid (*7*), algae, and fulvic acid (*8*). It has been reported that DBAN and DCAN may also be formed *in vivo* from hypochlorite (*9*).

Exposure to DHAN may not be limited to consumption of drinking water. For example, Trehy and Bieber (*7*) cite two proposed uses of DCAN: as an insecticide for grains (*10*) and as a biological growth inhibitor in cooling towers (*11*).

Concern about the potential adverse health effects associated with DHAN was heightened by data from Simmon et al. (*12*) showing that DCAN tested positive in the *Salmonella typhimurium* TA 100 assay (but did not increase mitotic recombination in *Saccharomyces cerevisiae*). Bull (*13*) and Meier et al. (*14*) extended these findings and reported that DCAN tested positive in three Salmonella tester strains (TA 98, TA 100, and TA 1535), but the relatively high cytotoxicity of DBAN compromised the *in vitro* mutagenicity testing (*13, 14*). DBAN tested positive, however, in a SENCAR mouse skin initiation-phorbol promotion model, which generated inconclusive data on DCAN (*13*). On the other hand, Kraybill (*15, 16*) identified DCAN as a mutagen or suspected mutagen in drinking water in the United States.

A review of the literature failed to identify toxicity data for DHAN. Since data were lacking on these compounds and they were of interest to the U.S. Environmental Protection Agency (EPA) because of their presence in drinking water, the following studies were undertaken as a joint effort to provide the needed toxicological information.

*Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.
†Study Director.
‡U.S. Environmental Protection Agency, 26 W. St. Clair St., Cincinnati, OH 45268.

Table 1. A series of nitriles.

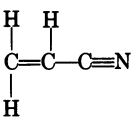
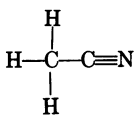
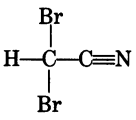
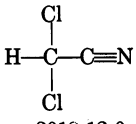
	Acrylonitrile	Acetonitrile	Dibromoacetonitrile	Dichloroacetonitrile
Structure				
CAS No.	107-13-1	75-05-8	3252-43-5	3018-12-0
Molecular weight	53.06	41.05	198.86	109.94
Boiling point, °C	77.5–79	81.6	67–69	112–113

Table 2. Acute oral toxicity protocol.

Animals
- Adult male and female CD-1 ICR mice; healthy, acclimated, randomized
- Adult male and female CD rats; healthy, acclimated, randomized
- Overnight (18 hr) fast (water, no food)
- 10 males, 10 females per dosage level; five levels
- Housing: one animal per plastic shoebox cage with hardwood sawdust bedding
- Feed: Purina Rodent Chow No. 5001; deionized water (in bottles)
- Environmental conditions:
  - Rooms: 22 ± 2°C; relative humidity: 40–60%
  - Light cycle: 12 light/12 dark (7:00 a.m.–7:00 p.m.)

Test material
- Identity confirmed by GC-MS
- Preparation: day of administration
- Administration: gavage (stomach tube)
- Volume: 10 mL/kg body weight

Specific conditions
- Food withheld for 2 hr after dosing
- Continuous observations for 1 hour after dosing, hourly for next 4 hr and twice daily for the next 14 days
- Cageside observations: include changes in behavior, respiration, circulation, skin and fur, eyes, mucous membranes; evidence of tremors or convulsions, lethargy, sleep, coma, salivation, diarrhea; time of onset of changes and duration; time of death
- All animals that died during the observation period and the survivors were necropsied

Calculations of LD_{50}:
- Litchfield-Wilcoxon (*19*)

Table 3. Short-term (14-days) repeated oral dosing protocol.

Animals
- Adult male and female CD rats; healthy, acclimated, randomized
- 10 males, 10 females per group
- Housing: One animal per stainless steel suspended cage
- Feed: Purina Rodent Chow No. 5001; automatic watering
- Environmental conditions:
  - Rooms: 22 ± 2°C; relative humidity: 40–60%
  - Light cycle: 12 light/12 dark (7:00 a.m.–7:00 p.m.)

Test material
- Identity confirmed by GC-MS
- Preparation: day of administration
- Vehicle: corn oil
- Administration: gavage (stomach tube)
- Volume: 10 mL/kg body weight

Specific conditions
- Groups: controls: naive, vehicle; three dosage levels
- Cageside observations: twice daily; behavior, morbidity, mortality; exceptions noted
- Body weights: onset, days 4, 7, 10, 13, and at sacrifice
- Hematology: WBC (total, differential); RBC; platelets; hematocrit; (HCrt)
- Serum chemistries: serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), 5′-nucleotidase, blood urea nitrogen (BUN), bilirubin, protein, albumin, globulin, glucose, cholesterol, creatinine, phosphorus (P), calcium (C), chlorine (Cl)
- Urinalyses: pH, protein, glucose, ketone, blood (Labstix)
- Necropsy: gross observations; organ weights
- Statistical analysis: appropriate tests including Bartlett's test for homogeneity, parametric ANOVA, Dunnett's multirange test, Wilcoxon nonparametric test; significance: $p \leq 0.05$

Materials and Methods

Materials and methods are summarized in Tables 1 through 4. The protocols are consistent with those proposed by the Food and Drug Administration (FDA) (*17*) and the Organization for Economic Cooperation and Development (OECD) (*18*).

Sprague-Dawley rats were supplied by Charles River Breeding Laboratories (N. Wilmington, MA). DCAN was obtained from ICN Pharmaceuticals, Inc., K & K Labs (Plainview, NY) (Lot No. 14128) and had a purity of 98%. Identity and purity were confirmed by gas chromatography-mass spectrometry (GC-MS). DBAN was obtained from Aldrich Chemical Co. (Milwaukee, WI) (Lot No. 1201 MJ) and had a purity of 97%. Identity and purity were confirmed by GC-MS.

Necropsy

The rats were weighed and then anesthetized with ether; blood was collected by cardiac puncture into 3.0% sodium citrate (1:10 citrate to blood) for the hematology studies and into uncitrated tubes for the serum chemistries. Gross pathological examination was performed, followed by removal and weighing of selected organs. All tissues were preserved in 10% neutral buffered formalin for subsequent histopathological examination.

Table 4. Subchronic (90-days) oral toxicity protocol.

Animals
- Adult male and female CD rats; healthy, acclimated, randomized
- 20 males, 20 females per group
- Housing: One animal per stainless steel suspended cage
- Feed: Purina Rodent Chow No. 5001; automatic watering
- Environmental conditions:
  - Rooms: 22 ± 2°C; relative humidity: 40–60%
  - Light cycle: 12 light/12 dark (7:00 a.m.–7:00 p.m.)

Test material:
- Identity confirmed by GC-MS
- Preparation: day of administration
- Vehicle: corn oil
- Administration: gavage (stomach tube)
- Volume: 10 mL/kg body weight

Specific conditions:
- Groups: controls: naive, vehicle; 3 dosage levels
- Cageside observations: twice daily; behavior, morbidity, mortality, exceptions noted
- Body weights: onset, once weekly, and at necropsy
- Hematology: WBC (total, differential); RBC; platelets; HCrt; Hb;
- Serum chemistries: SGPT, SGOT, ALP, 5′-nucleotidase, BUN, bilirubin, protein, albumin, globulin, glucose, cholesterol, creatinine, P, Ca, Cl
- Urinalyses: pH, protein, glucose, ketone, blood (Labstix)
- Necropsy: gross observations; organ weights
- Statistical analysis: appropriate tests including Bartlett's test for homogeneity, parametric ANOVA, Dunnett's multirange test, Wilcoxon nonparametric test; significance: $p \leq 0.05$

Hematology

A Coulter counter (Model Z81) was used to determine leukocyte, erythrocyte, and platelet numbers. Microhematocrits were determined, and hemoglobin was assayed as cyanomethemoglobin. Leukocyte differentials were evaluated by the classic Wright's Giemsa staining procedure. Prothrombin times and plasma fibrinogen levels were determined using reagents from Dade Diagnostics, Inc. (Miami, FL).

Serum Enzyme Chemistry

Serum enzyme levels and calcium and phosphorus concentrations were determined by using an Abbott Bichromatic Analyzer and diagnostic chemistry kits from Abbott Labs (N. Chicago, IL) and Sigma Chemical Co. (St. Louis, MO). Chloride levels were determined by using a Buchler chloridometer (Model 4-2500, Buchler Instruments, Fort Lee, NJ).

Table 5. Acute oral toxicity of haloacetonitriles in rats and mice[a]

	Acute oral LD_{50}, mg/kg			
	Rats		Mice	
	Male	Female	Male	Female
Dibromoacetonitrile	245 (210–286)[b]	361 (320–410)	289 (253–324)	303 (269–342)
Dichloroacetonitrile	339 (298–387)	330 (300–500)	270 (241–303)	279 (263–296)

[a]By gavage; corn oil vehicle.
[b]Method of Litchfield and Wilcoxon (*19*); LD_{50} ± confidence limits.

Urinalysis

Urinalysis was performed by using Labstix Reagent Strips from Miles Laboratories, Inc. (Elkhart, IN).

Statistical Evaluation

All data were subjected to an analysis of variance and test for homogeneity, as well as a Dunnett's *t*-test, and nonhomogenous data were subjected to a Wilcoxon rank sum test. Those values which differed from the vehicle group at $p \leq 0.05$ were considered significant.

Results

Acute Oral Toxicity of DBAN and DCAN

Acute oral toxicity data are summarized in Table 5. The LD_{50} values were calculated using the method of Litchfield and Wilcoxon (*19*). Ataxia, depressed respiration, depressed activity, and coma preceded death. No consistent, compound-related, gross pathological effects were observed at necropsy.

Toxicity of DBAN

14-Day Repeated Dosing. The body weight data, which are summarized in Figures 1 and 2, show that the depression in body weight gain was dose-dependent. There was 100% mortality at 180 mg/kg/day; all males were dead by day 4 and all females by day 7. At 90 mg/kg/day, 40% of males and 20% of females were dead by day 14.

No significant, consistent, compound-related and dose-dependent adverse effects were apparent in any of the hematological or urinary parameters measured, although a trend toward higher values for hemoglobin, total red blood count (RBC) and white blood count (WBC) and fibrinogen was observed in all treated animals (Tables 6 and 7). Additionally, no consistent, significant, compound-related adverse effects were observed in any of the serum chemistry parameters measured, which are summarized in Tables 6 and 7. Organ weights and ratios are summarized in Tables 8 and 9. No remarkable findings were observed at necropsy. Spleen and thymus in males and liver, lungs, and thymus in females were affected only at the highest dose. The significance of these effects in the absence of appropriate biochemical findings is unclear at this time.

90-Day Subchronic Study. The body weight data, summarized in Figures 3 and 4, show that body weight gain was significantly depressed only in males at the highest dose tested (45 mg/kg/day), confirming the apparent greater sensitivity of the male rat to the effects

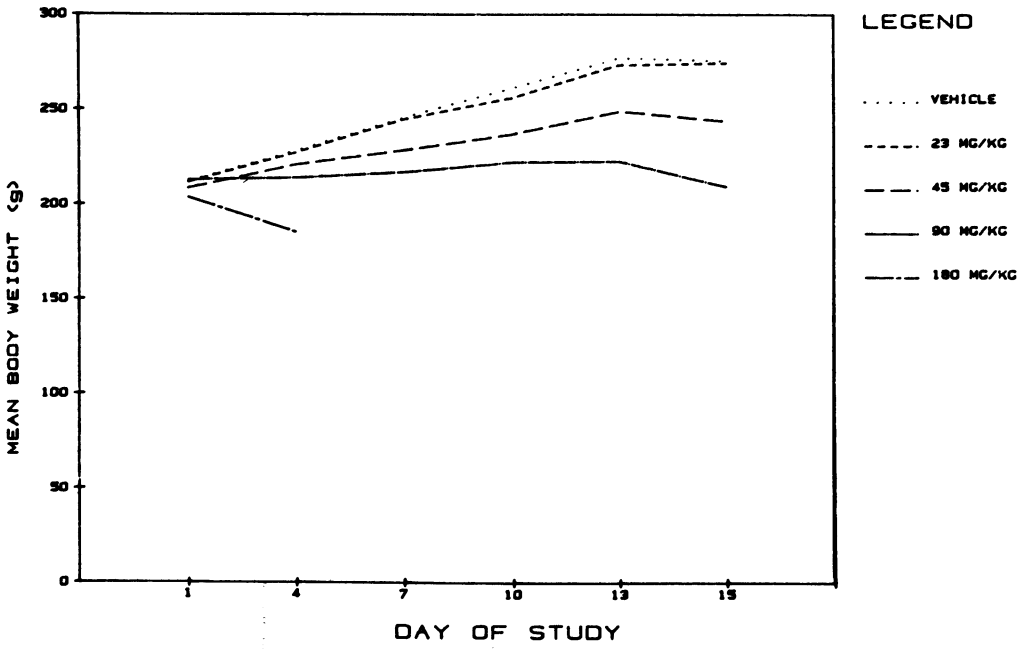


FIGURE 1. Mean body weights of male CD rats exposed to dibromoacetonitrile for 14 days.

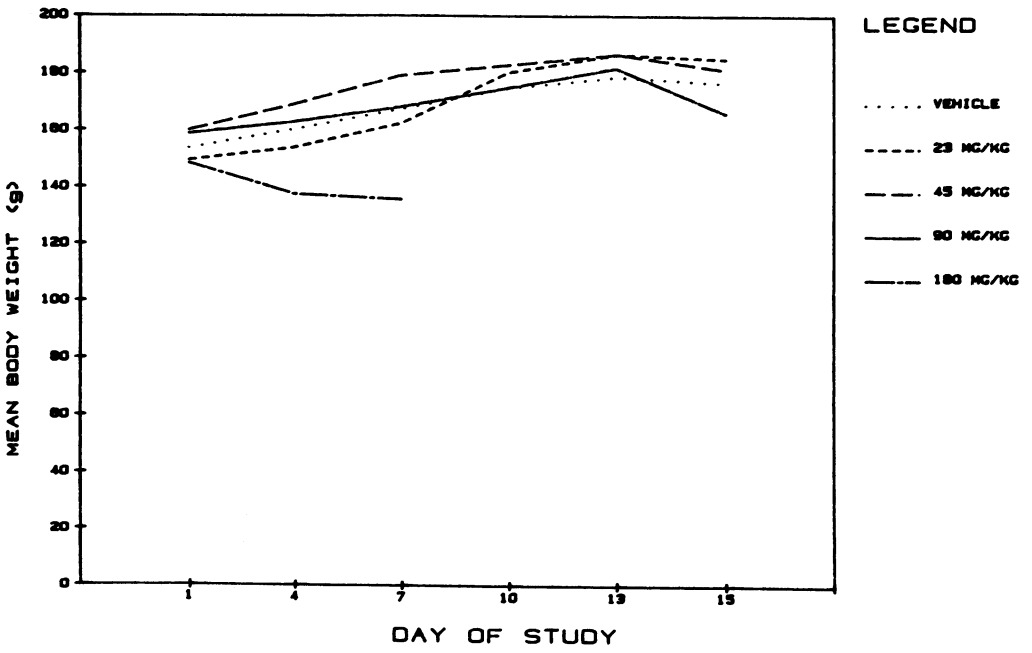


FIGURE 2. Mean body weights of female CD rats exposed to dibromoacetonitrile for 14 days.

of DBAN observed in the acute and 14-day repeated dosing study. Compound-related mortality was 5% (M) and 10% (F) at 45 mg/kg and 0% (M) and 10% (F) at 23 mg/kg during the 90-day study. No consistent, compound-related adverse effects were observed in any of the hematological or urinary parameters measured (Tables 10–15). Additionally, no significant, consistent, compound-related and dose-dependent adverse effects were observed in the serum chemistry parameters measured, which are summarized in Tables 10 through

Table 6. Serum chemistry mean values for CD male rats exposed to dibromoacetonitrile for 14 days.[a]

Parameter	Vehicle (corn oil)	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg	DBAN dose 90 mg/kg	DBAN dose 180 mg/kg
SGPT, IU/L	168 ± 55	75 ± 9	76 ± 11	77 ± 19	—[b]
SGOT, IU/L	339 ± 105	142 ± 14	172 ± 26	273 ± 81	—
ALP, IU/L	360 ± 41	466 ± 33	247 ± 29	187 ± 8*	—
5'-Nucleotidase, IU/L	18 ± 3	17 ± 1	13 ± 2	14 ± 1	—
Protein, g/dL	6.3 ± 0.1	6.5 ± 0.1	6.2 ± 0.1	4.7 ± 0.4*	—
Albumin, g/dL	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.1	2.9 ± 0.3*	—
Globulin, g/dL	2.3 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	1.8 ± 0.1*	—
Alb/globulin ratio	1.8 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	1.6 ± 0.2	—
Glucose, mg/dL	265 ± 47	212 ± 41	217 ± 40	130 ± 1	—
Cholesterol, mg/dL	66 ± 2	64 ± 2	74 ± 4	95 ± 5*	—
Bilirubin, mg/dL	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	—
BUN, mg/dL	15 ± 1	13 ± 1	10 ± 1	18 ± 6	—
Creatinine, mg/dL	1.3 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.0*	—
BUN/creatinine ratio	13 ± 2	16 ± 2	12 ± 1*	24 ± 8	—
Calcium mg/dL	11.3 ± 0.4	12.5 ± 0.6	11.8 ± 0.4	9.6 ± 0.6	—
Phosphorus, mg/dL	12.0 ± 0.4	11.2 ± 0.4	11.3 ± 0.5	7.6 ± 0.7*	—
Chloride, mEq/L	99 ± 2	99 ± 2	99 ± 1	100 ± 2	—

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).
[b]No survivors.

Table 7. Serum chemistry mean values for CD female rats exposed to dibromoacetonitrile for 14 days.[a]

Parameter	Vehicle (corn oil)	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg	DBAN dose 90 mg/kg	DBAN dose 180 mg/kg
SGPT, IU/L	74 ± 6	62 ± 8	74 ± 14	68 ± 11	—[b]
SGOT, IU/L	178 ± 32	138 ± 9	182 ± 21	161 ± 15	—
ALP, IU/L	236 ± 26	299 ± 29	260 ± 24	179 ± 34	—
5'-Nucleotidase, IU/L	25 ± 4	22 ± 2	28 ± 2	18 ± 4	—
Protein, g/dL	6.7 ± 0.1	6.2 ± 0.3	6.2 ± 0.1	5.3 ± 0.2*	—
Albumin, g/dL	4.3 ± 0.2	4.0 ± 0.1	4.2 ± 0.1	3.5 ± 0.1*	—
Globulin, g/dL	2.5 ± 0.1	2.2 ± 0.2	2.1 ± 0.1	1.8 ± 0.1*	—
Alb/globulin ratio	1.7 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	—
Glucose, mg/dL	212 ± 17	171 ± 18	197 ± 22	138 ± 17	—
Cholesterol, mg/dL	66 ± 4	58 ± 6	66 ± 6	71 ± 3	—
Bilirubin, mg/dL	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	—
BUN, mg/dL	15 ± 2	13 ± 2	13 ± 1	15 ± 2	—
Creatinine, mg/dL	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.0*	—
BUN/creatinine ratio	12 ± 2	15 ± 4	14 ± 2	19 ± 3	—
Calcium, mg/dL	11.9 ± 0.4	11.1 ± 0.3	12.1 ± 0.5	11.1 ± 0.3	—
Phosphorus, mg/dL	11.6 ± 0.1	10.1 ± 0.6	10.8 ± 0.5	9.3 ± 0.7*	—
Chloride, mEq/L	98 ± 1.4	102 ± 0.7	100 ± 1.2	101 ± 0.7	—

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).
[b]No survivors.

15, although there are suggestions that the liver could be affected. Organ weights and ratios are summarized in Tables 16 and 17. No remarkable findings were apparent at necropsy. Possible target organs were determined and included the thymus, liver, and kidneys. Supporting biochemical evidence for these target organs was, however, not detectable. The apparent no-observed adverse-effect level (NOAEL) for DBAN, based on these 90-day data, was determined to be 23 mg/kg/day.

Table 8. Organ weights and ratios of male CD rats exposed to dibromoacetonitrile by gavage for 14 days.[a]

Organ	Vehicle (corn oil)	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg	DBAN dose 90 mg/kg	DBAN dose 180 mg/kg
Body weight, g	275.5 ± 3.2	274.6 ± 5.5	243.7 ± 4.6*	209.4 ± 10.1*	—
Brain, mg	1810 ± 50	1770 ± 60	1760 ± 30	1660 ± 50	—
% body weight	0.66 ± 0.02	0.65 ± 0.03	0.73 ± 0.02*	0.80 ± 0.06*	—
Liver, mg	12910 ± 340	11600 ± 1240	11470 ± 1200	10180 ± 1890	—
% body weight	4.69 ± 0.15	4.25 ± 0.45	4.71 ± 0.49	4.72 ± 0.83	—
% brain weight	719 ± 24	668 ± 72	659 ± 73	630 ± 123	—
Spleen, mg	640 ± 30	590 ± 50	560 ± 50	420 ± 50*	—
% body weight	0.23 ± 0.01	0.21 ± 0.02	0.23 ± 0.02	0.20 ± 0.02	—
% brain weight	35.8 ± 1.7	33.2 ± 2.6	31.8 ± 2.8	25.5 ± 3.4*	—
Lungs, mg	1690 ± 100	1490 ± 90	1440 ± 70	1300 ± 140*	—
% body weight	0.61 ± 0.03	0.54 ± 0.04	0.59 ± 0.03	0.62 ± 0.06	—
% brain weight	94.0 ± 6.0	84.6 ± 5.0	81.4 ± 4.1	80.2 ± 11.3	—
Thymus, mg	510 ± 30	520 ± 40	460 ± 20	240 ± 60*	—
% body weight	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.11 ± 0.03*	—
% brain weight	28.7 ± 1.9	29.7 ± 2.7	26.1 ± 1.8	15.2 ± 4.3*	—
Kidneys, mg	2500 ± 100	2390 ± 90	2080 ± 60*	2130 ± 150*	—
% body weight	0.91 ± 0.05	0.87 ± 0.03	0.85 ± 0.02	1.02 ± 0.06	—
% brain weight	139.6 ± 6.9	135.5 ± 3.6	118.1 ± 4.1*[b]	130.2 ± 12.1	—
Testes, mg	2700 ± 90	2710 ± 70	2670 ± 60	2690 ± 60	—
% body weight	0.99 ± 0.04	0.99 ± 0.03	1.10 ± 0.03	1.30 ± 0.07*	—
% brain weight	150.5 ± 5.5	154.9 ± 6.0	152.0 ± 5.5	163.0 ± 5.2	—

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).
[b]No survivors.

Table 9. Organ weights and ratios of female CD rats exposed to dibromoacetonitrile by gavage for 14 days.[a]

Organ	Vehicle (corn oil)	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg	DBAN dose 90 mg/kg	DBAN dose 180 mg/kg
Body weight, g	176.5 ± 6.8	184.9 ± 2.4	181.1 ± 2.8	165.9 ± 5.4	—[b]
Brain, mg	1670 ± 40	1680 ± 40	1640 ± 40	1690 ± 60	—
% body weight	0.96 ± 0.04	0.91 ± 0.03	0.91 ± 0.03	1.03 ± 0.04	—
Liver, mg	8590 ± 260	9650 ± 250*	9730 ± 200*	10500 ± 440*	—
% body weight	4.89 ± 0.12	5.22 ± 0.11	5.39 ± 0.15	6.34 ± 0.24*	—
% brain weight	515 ± 20	577 ± 24	598 ± 18*	622 ± 23*	—
Spleen, mg	480 ± 30	450 ± 20	520 ± 30	420 ± 40	—
% body weight	0.27 ± 0.01	0.24 ± 0.01	0.29 ± 0.02	0.26 ± 0.02	—
% brain weight	28.6 ± 1.7	27.0 ± 1.0	31.6 ± 1.5	25.0 ± 2.3	—
Lungs, mg	1580 ± 190	1420 ± 90	1250 ± 60	1090 ± 60*	—
% body weight	0.88 ± 0.08	0.77 ± 0.05	0.69 ± 0.04	0.66 ± 0.04*	—
% brain weight	94.1 ± 10.7	84.4 ± 5.9	76.3 ± 2.9	65.0 ± 5.0*	—
Thymus, mg	400 ± 30	440 ± 30	400 ± 10	210 ± 20*	—
% body weight	0.23 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.13 ± 0.01*	—
% brain weight	24.4 ± 2.3	26.3 ± 1.6	24.4 ± 1.0	12.9 ± 1.0*	—
Kidneys, mg	1560 ± 60	1640 ± 40	1620 ± 40	1530 ± 50	—
% body weight	0.89 ± 0.02	0.89 ± 0.02	0.89 ± 0.03	0.92 ± 0.03	—
% brain weight	94.0 ± 4.8	97.8 ± 3.2	99.5 ± 3.9	90.9 ± 4.2	—
Ovaries, mg	110 ± 10	140 ± 10	140 ± 10	120 ± 20	—
% body weight	0.06 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	—
% brain weight	6.7 ± 0.5	8.2 ± 0.7	8.5 ± 1.0	6.9 ± 0.8	—

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).
[b]No survivors.

Toxicity of DCAN

14-Day Repeated Dosing. The body weight data, summarized in Figures 5 and 6, show that the depression in body weight gain was dose-dependent. There was no mortality, and no consistent, significant, compound-related and dose-dependent adverse effects were observed in any of the hematological or urinary param-

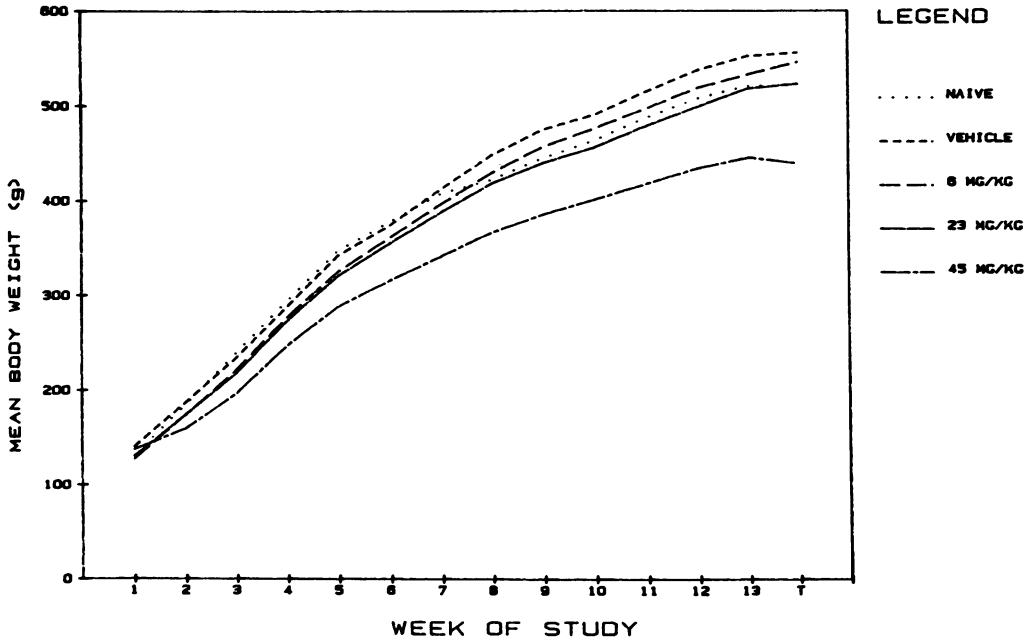


FIGURE 3. Mean body weights of male CD rats exposed to dichloroacetonitrile for 14 days.

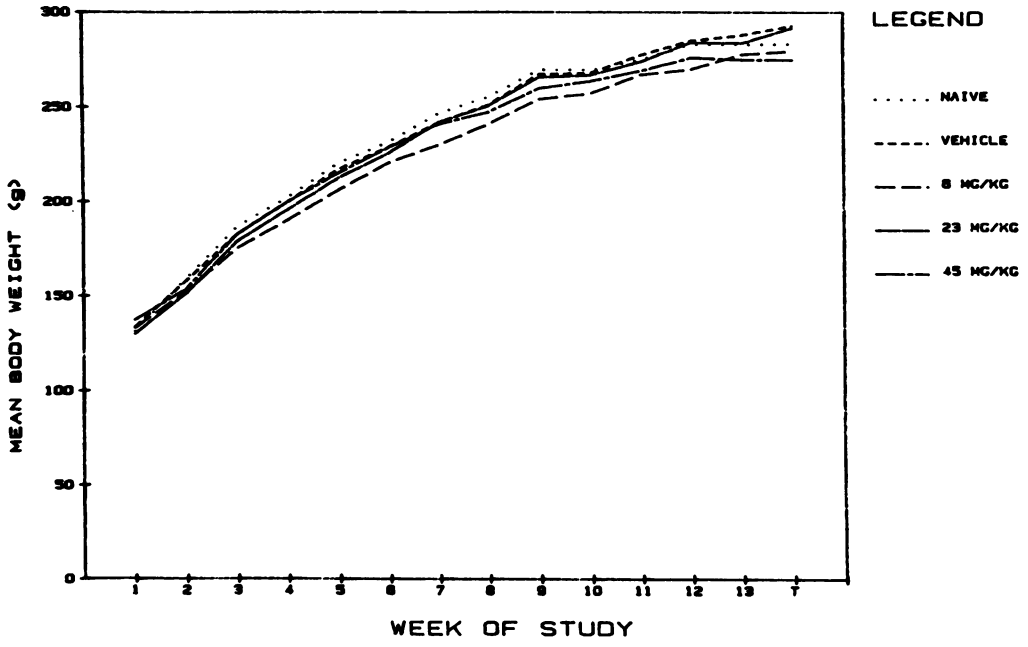


FIGURE 4. Mean body weights of female CD rats exposed to dichloracetonitrile for 14 days.

eters measured, although a trend toward elevated RBC and WBC was noted in all treated animals. The serum chemistry values, summarized in Tables 18 and 19, revealed no consistent, significant, compound-related and dose-dependent adverse effects. Organ weights and ratios are summarized in Tables 20 and 21. No remarkable findings were observed at necropsy, although most organ weights and ratios were significantly lower at the

Table 10. Serum chemistry mean values for CD male rats exposed to dibromoacetonitrile at day 30 and 31 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
SGPT, IU/L	80 ± 3	65 ± 3	51 ± 2	54 ± 3	70 ± 13
SGOT, IU/L	208 ± 16	242 ± 23	194 ± 17	227 ± 14	222 ± 41
ALP, IU/L	293 ± 20	319 ± 22	281 ± 26	310 ± 30	287 ± 26
5'-Nucleotidase, IU/L	16 ± 3	20 ± 5	16 ± 4	19 ± 5	16 ± 4
Protein, g/dL	7.4 ± 0.1	7.8 ± 0.2	8.1 ± 0.2	8.1 ± 0.1	8.0 ± 0.2
Albumin, g/dL	4.8 ± 0.1	5.1 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.2 ± 0.1
Globulin, g/dL	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	2.8 ± 0.2
Alb/globulin ratio	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.3	2.4 ± 0.3	2.0 ± 0.2
Glucose, mg/dL	137 ± 8	131 ± 7	144 ± 8	139 ± 12	161 ± 10
Cholesterol, mg/dL	78 ± 3	81 ± 4	77 ± 7	79 ± 6	71 ± 0.1
Bilirubin, mg/dL	0.4 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
BUN, mg/dL	20 ± 1*	16 ± 1	17 ± 1	16 ± 1	14 ± 1
Creatinine, mg/dL	1.2 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.1
BUN/creatinine ratio	17 ± 1*	10 ± 0.6	13 ± 1	15 ± 2*	11 ± 0.7
Calcium, mg/dL	11.8 ± 0.3	12.0 ± 0.6	12.5 ± 0.4	12.6 ± 0.4	11.8 ± 0.6
Phosphorus, mg/dL	8.6 ± 0.3	9.2 ± 0.6	8.7 ± 0.3	9.3 ± 0.3	9.3 ± 0.5
Chloride, mEq/L	101 ± 1	103 ± 1	101 ± 1	100 ± 1	101 ± 1

[a]All data expressed as mean ± SEM.
[b]Significantly different from vehicle control ($p \leq 0.05$).

Table 11. Serum chemistry mean values for CD male rats exposed to dibromoacetonitrile at day 59 and 60 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
SGPT, IU/L	45 ± 4	50 ± 2	55 ± 6.5	43 ± 2.7	56 ± 8.4
SGOT, IU/L	204 ± 32	229 ± 32	221 ± 32	195 ± 23	202 ± 23
ALP, IU/L	174 ± 24	220 ± 16	192 ± 24	226 ± 25	177 ± 19
5'-Nucleotidase, IU/L	14 ± 1	16 ± 1	14 ± 1	15 ± 1	14 ± 1
Protein, g/dL	6.8 ± 0.2	7.1 ± 0.3	7.3 ± 0.2	7.7 ± 0.2	7.1 ± 0.2
Albumin, g/dL	5.0 ± 0.1	5.2 ± 0.1	5.4 ± 0.2	5.7 ± 0.1	5.4 ± 0.2
Globulin, g/dL	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.3	2.0 ± 0.2	1.7 ± 0.3
Alb/globulin ratio	2.8 ± 0.6	3.2 ± 1.3	3.3 ± 2.1	2.9 ± 1.3	3.5 ± 2.1
Glucose, mg/dL	148 ± 5	164 ± 8	148 ± 5	148 ± 7	154 ± 7
Cholesterol mg/dL	65 ± 4	81 ± 7	77 ± 7	72 ± 4	72 ± 5
Bilirubin, mg/dL	0.3 ± 0.0*	0.8 ± 0.4	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.1
BUN, mg/dL	21 ± 1*	18 ± 1	16 ± 1	19 ± 1	16 ± 1
Creatinine, mg/dL	1.1 ± 0.0	1.1 ± 0.2	1.4 ± 0.1	1.2 ± 0.1	0.8 ± 0.2
BUN/creatinine ratio	20 ± 1	27 ± 1	12 ± 1	17 ± 1	25 ± 4
Calcium, mg/dL	11.0 ± 0.2	11.9 ± 0.4	12.3 ± 0.3	12.5 ± 0.3	11.7 ± 0.4
Phosphorus, mg/dL	7.1 ± 0.2	7.7 ± 0.3	7.9 ± 0.5	8.3 ± 0.4	9.0 ± 0.3
Chloride, mEq/L	101 ± 1	101 ± 1	101 ± 1	101 ± 1	101 ± 1

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).

highest dose tested (90 mg/kg/day). The significance of these findings is unclear.

90-Day Subchronic Study. The body weight data, summarized in Figures 7 and 8, show that body weight gain was significantly depressed in male and female rats at 65 mg/kg/day. The following mortality was observed: at 65 mg/kg, 50% of males and 25% of females had died by the completion of the study; at 33 mg/kg, 10% of males and 5% of females had died; and at 8 mg/kg, 5% of males had died. No consistent, compound-related and

Table 12. Serum chemistry mean values for CD male rats exposed to dibromoacetonitrile at termination of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
SGPT, IU/L	58 ± 14	44 ± 4	69 ± 3	42 ± 4	44 ± 5
SGOT, IU/L	200 ± 31	176 ± 12	245 ± 52	190 ± 30	181 ± 13
ALP, IU/L	159 ± 16	191 ± 16	171 ± 28	192 ± 23	157 ± 16
5'-Nucleotidase, IU/L	14 ± 1	15 ± 1	17 ± 2	16 ± 1	13 ± 1
Protein, g/dL	6.7 ± 0.1*	8.2 ± 0.4	8.2 ± 0.3	8.1 ± 0.2	7.0 ± 0.2*
Albumin, g/dL	5.5 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.8 ± 0.1*	5.8 ± 0.1*
Globulin, g/dL	1.2 ± 0.1*	2.9 ± 0.5	2.9 ± 0.4	2.3 ± 0.2	1.2 ± 0.1*
Alb/globulin ratio	4.6 ± 0.2	2.5 ± 0.6	2.3 ± 0.3	2.8 ± 0.4	5.8 ± 0.8
Glucose, mg/dL	138 ± 9	148 ± 7	139 ± 7	143 ± 8	134 ± 5
Cholesterol, mg/dL	74 ± 5	73 ± 5	70 ± 5	69 ± 4	77 ± 5
Bilirubin, mg/dL	0.3 ± 0.0*	0.6 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.0
BUN, mg/dL	22 ± 1*	17 ± 1	15 ± 1	17 ± 1	18 ± 1
Creatinine, mg/dL	1.1 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	1.3 ± 0.0	1.0 ± 0.0
BUN/creatinine ratio	21 ± 1	17 ± 1	17 ± 2	14 ± 0	19 ± 1
Calcium, mg/dL	10.4 ± 0.1	11.3 ± 0.5	11.7 ± 0.2	12.8 ± 0.2*	10.9 ± 0.2
Phosphorus, mg/dL	6.0 ± 0.1	6.4 ± 0.4	6.9 ± 0.4	6.7 ± 0.2	6.5 ± 0.2
Chloride, mEq/L	100 ± 1	102 ± 1	101 ± 1	102 ± 1	102 ± 1

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).

Table 13. Serum chemistry mean values for CD female rats exposed to dibromoacetonitrile at day 30 and 31 of subchronic study.

Parameter	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
SGPT, IU/L	83 ± 6*	49 ± 3	52 ± 3	41 ± 2	37 ± 2
SGOT, IU/L	194 ± 13	199 ± 13	201 ± 25	129 ± 10*	152 ± 17
ALP, IU/L	184 ± 14	186 ± 15	195 ± 12	208 ± 25	198 ± 22
5'-Nucleotidase, IU/L	27 ± 3	27 ± 2	29 ± 2	35 ± 5	28 ± 3
Protein, g/dL	7.8 ± 0.1	7.6 ± 0.2	7.9 ± 0.1	7.8 ± 0.2	7.7 ± 0.2
Albumin, g/dL	5.3 ± 0.1	5.6 ± 0.2	5.9 ± 0.1	5.9 ± 0.1	6.1 ± 0.1*
Globulin, g/dL	2.5 ± 0.2	2.1 ± 0.3	2.0 ± 0.2	1.8 ± 0.3	1.6 ± 0.1
Alb/globulin ratio	2.2 ± 0.2	3.4 ± 0.7	3.1 ± 0.3	4.8 ± 2.1	3.4 ± 0.4
Glucose, mg/dL	133 ± 5	124 ± 4	136 ± 7	154 ± 5*	178 ± 7*
Cholesterol, mg/dL	102 ± 4	88 ± 3	82 ± 4	89 ± 6	65 ± 3*
Bilirubin, mg/dL	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.1
BUN, mg/dL	19 ± 1*	14 ± 1	14 ± 1	13 ± 1	16 ± 1
Creatinine, mg/dL	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.5 ± 0.2
BUN/creatinine ratio	15 ± 1	11 ± 1	12 ± 1	15 ± 2	12 ± 2
Calcium mg/dL	11.7 ± 0.2	11.2 ± 0.2	11.1 ± 0.2	12.0 ± 0.2	12.0 ± 0.4
Phosphorus, mg/dL	7.3 ± 0.3	8.0 ± 0.2	7.2 ± 0.2	8.2 ± 0.4	8.6 ± 0.7
Chloride, mEq/L	101 ± 0.7	100 ± 0.8	101 ± 0.9	102 ± 0.7	100 ± 0.8

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).

dose-dependent adverse effects were observed in any of the hematological and urinary parameters measured. The serum chemistry values, summarized in Tables 22–27, revealed few significant, consistent, compound-related and dose-dependent adverse effects. These effects included a lowering of cholesterol at the highest dose tested (65 mg/kg/day) and elevated levels of serum glutamic-pyruvic transaminase (SGPT) suggesting possible liver involvement. The significance of decreases in the activity of selected enzymes is unclear.

Table 14. Serum chemistry mean values for CD female rats exposed to dibromoacetonitrile at day 59 and 60 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
SGPT, IU/L	47 ± 3	44 ± 3	40 ± 5	40 ± 4	35 ± 2
SGOT, IU/L	203 ± 21	238 ± 34	201 ± 35	161 ± 18	241 ± 43
ALP, IU/L	135 ± 18	150 ± 9	162 ± 10	189 ± 23	203 ± 24
5'-Nucleotidase, IU/L	28 ± 2	23 ± 2	25 ± 2	28 ± 2	26 ± 2
Protein, g/dL	7.3 ± 0.1	7.3 ± 0.2	7.3 ± 0.2	7.0 ± 0.1	6.9 ± 0.2
Albumin, g/dL	5.4 ± 0.1	5.8 ± 0.2	6.1 ± 0.1	6.2 ± 0.1	6.0 ± 0.1
Globulin, g/dL	1.9 ± 0.1	1.5 ± 0.2	1.2 ± 0.2	0.8 ± 0.1*	0.9 ± 0.1
Alb/globulin ratio	2.9 ± 0.2	5.6 ± 1.7	6.1 ± 1.4	9.4 ± 3.7	6.4 ± 0.6
Glucose, mg/dL	169 ± 11	154 ± 7	161 ± 10	161 ± 12	168 ± 7
Cholesterol, mg/dL	83 ± 2	88 ± 5	83 ± 4	83 ± 5	63 ± 4*
Bilirubin, mg/dL	0.2 ± 0.0*	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
BUN, mg/dL	20 ± 1*	15 ± 1	15 ± 1	16 ± 1	13 ± 1
Creatinine, mg/dL	1.2 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	0.8 ± 0.1*
BUN/creatinine ratio	18 ± 2*	11 ± 1	11 ± 0	17 ± 2*	18 ± 2*
Calcium, mg/dL	10.9 ± 0.2	11.4 ± 0.2	11.4 ± 0.1	11.8 ± 0.1	11.3 ± 0.2
Phosphorus, mg/dL	6.2 ± 0.3	6.3 ± 0.3	6.0 ± 0.4	6.4 ± 0.3	6.9 ± 0.3
Chloride, mEq/L	102 ± 1	103 ± 1	115 ± 12	101 ± 1	101 ± 1

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).

Table 15. Serum chemistry mean values for CD female rats exposed to dibromoacetonitrile at termination of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
SGPT, IU/L	56 ± 6	46 ± 3	35 ± 3	37 ± 3	32 ± 3*
SGOT, IU/L	183 ± 21	207 ± 31	169 ± 31	174 ± 10	161 ± 11
ALP, IU/L	143 ± 19	134 ± 11	130 ± 9	138 ± 19	208 ± 32*
5'-Nucleotidase, IU/L	29 ± 2	26 ± 2	26 ± 2	24 ± 2	19 ± 1*
Protein, g/dL	6.8 ± 0.1*	7.3 ± 0.2	7.5 ± 0.2	7.1 ± 0.2	6.9 ± 0.2
Albumin, g/dL	5.8 ± 0.1	6.1 ± 0.1	6.6 ± 0.1*	6.1 ± 0.1	5.6 ± 0.1*
Globulin, g/dL	1.0 ± 0.1	1.2 ± 0.1	0.9 ± 0.2	1.1 ± 0.1	1.3 ± 0.2
Alb/globulin ratio	6.4 ± 0.5	5.7 ± 0.6	9.0 ± 1.3*	6.3 ± 0.8	5.3 ± 0.8
Glucose, mg/dL	146 ± 10	145 ± 8	147 ± 5	135 ± 10	130 ± 7
Cholesterol, mg/dL	83 ± 3	80 ± 4	80 ± 6	78 ± 4	64 ± 3*
Bilirubin, mg/dL	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.7 ± 0.0*
BUN, mg/dL	21 ± 1*	18 ± 1	18 ± 1	18 ± 1	15 ± 1
Creatinine, mg/dL	1.3 ± 0.0	1.2 ± 0.0	1.3 ± 0.1	1.0 ± 0.1	1.0 ± 0.0*
BUN/creatinine ratio	16 ± 1	15 ± 1	14 ± 1	18 ± 2	15 ± 1
Calcium, mg/dL	10.6 ± 0.2	10.9 ± 0.2	11.4 ± 0.2	11.1 ± 0.2	10.7 ± 0.3
Phosphorus, mg/dL	5.0 ± 0.3	4.6 ± 0.2	4.8 ± 0.1	5.7 ± 0.2*	6.2 ± 0.3*
Chloride, mEq/L	101 ± 1	102 ± 1	101 ± 1	102 ± 1	101 ± 1

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).

Organ weights and ratios are summarized in Tables 28 and 29. No remarkable findings were observed at necropsy, although most organ weights and ratios were lower in males at 65 mg/kg, and livers appeared to be larger in treated females. The apparent NOAEL for DCAN, based on these 90-day data, was determined to be 8 mg/kg/day.

Discussion

Pereira et al. (*20*) studied the metabolism and excretion of haloacetonitriles (HAN) and reported that 7.7 to 12.8% of orally administered DBAN and DCAN was converted to thiocyanate and excreted in the urine. They also reported that some orally administered HAN

Table 16. Organ weights and ratios at termination of subchronic study of male CD rats exposed to dibromoacetonitrile by gavage.[a]

Organ	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
Body weight, g	521.7 ± 12.8	556.0 ± 12.7	545.9 ± 13.5	523.1 ± 11.4	438.6 ± 7.5*
Brain, mg	1970 ± 60	1960 ± 40	1980 ± 50	1950 ± 40	1930 ± 40
% body weight	0.38 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.38 ± 0.01	0.44 ± 0.01*
Liver, mg	19890 ± 670	21850 ± 990	21700 ± 680	22390 ± 840	19700 ± 670
% body weight	3.81 ± 0.08	3.93 ± 0.15	3.98 ± 0.08	4.27 ± 0.12	4.44 ± 0.13*
% brain weight	1033 ± 60	1117 ± 50	1114 ± 56	1157 ± 54	1022 ± 32
Spleen, mg	810 ± 30	840 ± 40	840 ± 30	780 ± 40	720 ± 40
% body weight	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
% brain weight	42.0 ± 2.2	42.8 ± 1.5	42.6 ± 1.8	40.1 ± 1.9	37.3 ± 2.0
Lungs, mg	3060 ± 110	3220 ± 140	2950 ± 90	3090 ± 110	2730 ± 90*
% body weight	0.59 ± 0.02	0.58 ± 0.03	0.54 ± 0.02	0.59 ± 0.02	0.63 ± 0.03
% brain weight	157.2 ± 6.9	164.4 ± 7.4	150.0 ± 4.9	158.3 ± 5.1	141.9 ± 4.5*
Thymus, mg	440 ± 20*	550 ± 30	500 ± 30	530 ± 20	430 ± 30*
% body weight	0.08 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.00	0.10 ± 0.01
% brain weight	32.7 ± 1.4*	28.4 ± 2.0	25.3 ± 1.4	27.5 ± 1.3	22.6 ± 1.7
Kidneys, mg	3770 ± 100	3670 ± 100	3560 ± 90	3620 ± 100	3160 ± 100*
% body weight	0.73 ± 0.01*	0.66 ± 0.02	0.66 ± 0.02	0.69 ± 0.01	0.72 ± 0.02
% brain weight	195.6 ± 9.9	188.2 ± 6.8	181.2 ± 5.9	186.8 ± 7.0	164.0 ± 4.7*
Testes, mg	3720 ± 60	3580 ± 50	3760 ± 70	3490 ± 60	3510 ± 150
% body weight	0.72 ± 0.02*	0.65 ± 0.01	0.69 ± 0.02	0.67 ± 0.02	0.80 ± 0.03*
% brain weight	192.7 ± 8.3	183.3 ± 4.6	191.8 ± 6.0	180.5 ± 5.7	182.8 ± 8.9

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$).

Table 17. Organ weights and ratios at termination of subchronic study of female CD rats exposed to dibromoacetonitrile by gavage.[a]

Organ	Naive	Vehicle (corn oil)	DBAN dose 6 mg/kg	DBAN dose 23 mg/kg	DBAN dose 45 mg/kg
Body weight, g	283.3 ± 7.7	292.9 ± 5.8	279.6 ± 7.3	291.7 ± 9.5	274.9 ± 7.2
Brain, mg	1840 ± 30	1830 ± 30	1810 ± 40	1760 ± 40	1760 ± 40
% body weight	0.66 ± 0.02	0.63 ± 0.02	0.65 ± 0.02	0.61 ± 0.01	0.64 ± 0.02
Liver, mg	11150 ± 420	11620 ± 370	11100 ± 360	12310 ± 330	11950 ± 390
% body weight	3.98 ± 0.17	3.98 ± 0.12	3.98 ± 0.10	4.25 ± 0.11	4.36 ± 0.12
% brain weight	603 ± 18	638 ± 21	619 ± 25	704 ± 21	688 ± 32
Spleen, mg	530 ± 30	510 ± 30	520 ± 20	550 ± 30	570 ± 40
% body weight	0.19 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
% brain weight	28.9 ± 1.3	27.9 ± 1.8	29.3 ± 1.3	31.2 ± 1.6	32.9 ± 2.4
Lungs, mg	1940 ± 80	2270 ± 150	2340 ± 160	2350 ± 140	2470 ± 170
% body weight	0.69 ± 0.03	0.78 ± 0.05	0.85 ± 0.07	0.81 ± 0.05	0.90 ± 0.06
% brain weight	105.9 ± 4.6	125.1 ± 8.5	129.9 ± 8.4	133.8 ± 6.7	142.3 ± 10.7
Thymus, mg	390 ± 30	400 ± 30	400 ± 30	340 ± 20	260 ± 30*
% body weight	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.12 ± 0.01	1.10 ± 0.01*
% brain weight	21.3 ± 1.6	21.5 ± 1.3	22.7 ± 1.9	19.5 ± 1.2	15.2 ± 1.4*
Kidneys, mg	2080 ± 80	2100 ± 50	1980 ± 60	2010 ± 700	1980 ± 70
% body weight	0.75 ± 0.04	0.72 ± 0.02	0.71 ± 0.02	0.70 ± 0.02	0.72 ± 0.02
% brain weight	112.5 ± 3.0	115.1 ± 3.1	110.2 ± 4.0	115.1 ± 4.0	113.8 ± 4.8
Ovaries, mg	160 ± 10	170 ± 10	170 ± 10	160 ± 10	170 ± 10
% body weight	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
% brain weight	8.9 ± 0.4	9.1 ± 0.5	9.2 ± 0.5	8.9 ± 0.5	9.4 ± 0.6

[a] All data expressed as mean ± SEM.
[a] Significantly different from vehicle control at ($p \leq 0.05$).

will inhibit hepatic dimethylnitrosamine demethylase (DMN-DM) activity. They concluded that HAN are converted to highly toxic metabolites. Daniel et al. (*21*) reported that HAN are direct-acting alkylating agents and could elicit liver and/or other organ toxicity, including carcinogenesis. These findings and those of Sim-

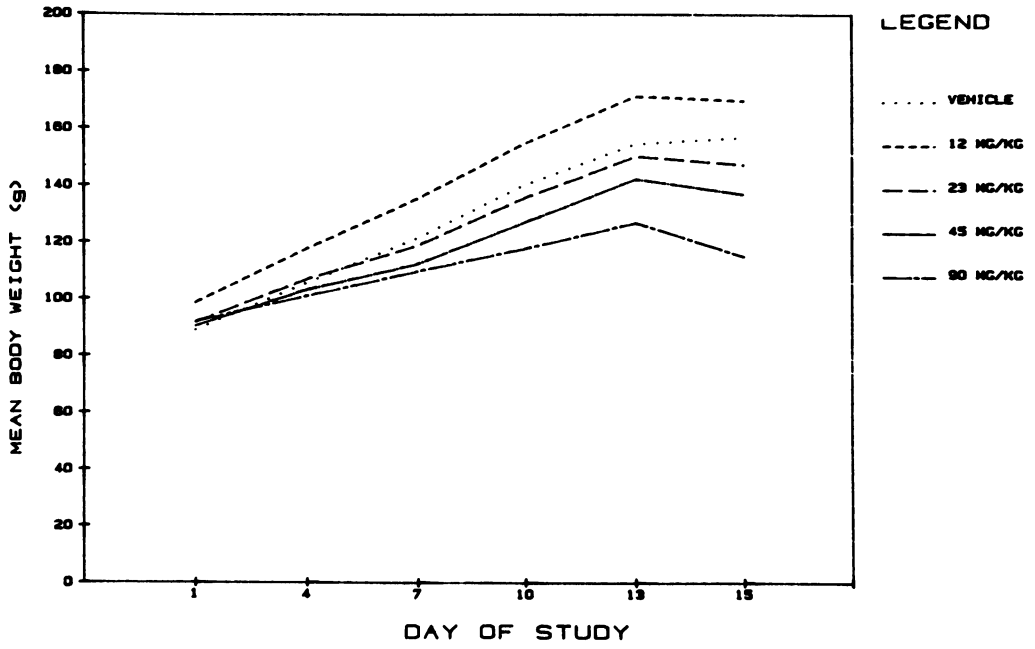


FIGURE 5. Weekly body weights of male CD rats exposed to dibromoacetonitrile for 13 weeks.

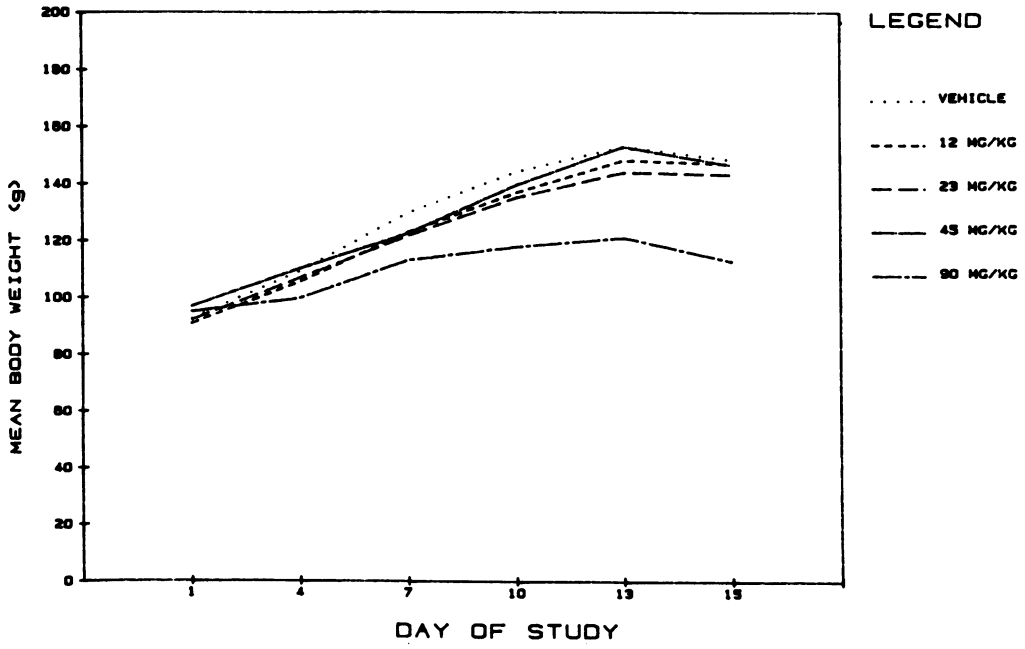


FIGURE 6. Weekly body weights of female CD rats exposed to dibromoacetonitrile for 13 weeks.

Table 18. Serum chemistry mean values for CD male rats exposed to dichloroacetonitrile for 14 days.[a]

Parameter	Vehicle (corn oil)	DCAN dose 12 mg/kg	DCAN dose 23 mg/kg	DCAN dose 45 mg/kg	DCAN dose 90 mg/kg
SGPT, IU/L	70 ± 3	71 ± 6	89 ± 22	87 ± 15	88 ± 14
SGOT, IU/L	167 ± 13	266 ± 22	213 ± 53	276 ± 40	233 ± 27
ALP, IU/L	457 ± 63	459 ± 39	384 ± 21	398 ± 50	712 ± 84*
5'-Nucleotidase, IU/L	14 ± 1	16 ± 1	14 ± 2	17 ± 2	23 ± 2*
Protein, g/dL	5.8 ± 0.2	6.0 ± 0.2	5.7 ± 0.2	5.5 ± 0.2	6.0 ± 0.1
Albumin, g/dL	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.8 ± 0.1*
Globulin, g/dL	1.4 ± 0.1	1.7 ± 0.1	1.4 ± 0.2	1.2 ± 0.2	1.2 ± 0.2
Alb/globulin ratio	3.1 ± 0.1	2.6 ± 0.1*	3.4 ± 0.5	3.8 ± 0.5	4.5 ± 0.7
Glucose, mg/dL	154 ± 11	129 ± 16	138 ± 6	134 ± 10	112 ± 16
Cholesterol, mg/dL	90 ± 5	93 ± 2	91 ± 6	80 ± 10	81 ± 11
Bilirubin, mg/dL	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0*	0.6 ± 0.1
BUN, mg/dL	17 ± 2	17 ± 2	15 ± 2	17 ± 2	15 ± 1
Creatinine, mg/dL	1.7 ± 0.2	1.4 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
BUN/creatinine ratio	11 ± 3	12 ± 1	11 ± 1	12 ± 2	11 ± 1
Calcium, mg/dL	11.4 ± 0.1	11.0 ± 0.2	11.3 ± 0.2	9.8 ± 1.5	11.7 ± 0.3
Phosphorus, mg/dL	12.3 ± 0.6	12.0 ± 0.8	12.5 ± 0.6	11.7 ± 0.5	12.9 ± 0.4
Chloride, mEq/L	100 ± 1	100 ± 1	101 ± 1	101 ± 2	101 ± 1

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control at ($p \leq 0.05$).

Table 19. Serum chemistry mean values for CD female rats exposed to dichloroacetonitrile for 14 days.[a]

Parameter	Vehicle (corn oil)	DCAN dose 12 mg/kg	DCAN dose 23 mg/kg	DCAN dose 45 mg/kg	DCAN dose 90 mg/kg
SGPT, IU/L	57 ± 6	66 ± 7	63 ± 4	63 ± 7	134 ± 38*
SGOT, IU/L	205 ± 8	198 ± 6	201 ± 33	181 ± 28	340 ± 96
ALP, IU/L	261 ± 24	381 ± 48	352 ± 45	384 ± 35*	651 ± 159*
5'-Nucleotidase, IU/L	23 ± 3	20 ± 2	16 ± 1	18 ± 1	24 ± 2
Protein, g/dL	6.4 ± 0.3	6.2 ± 0.2	6.4 ± 0.3	5.9 ± 0.2	6.1 ± 0.4
Albumin, g/dL	4.6 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	4.8 ± 0.2
Globulin, g/dL	1.8 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.2 ± 0.1	1.3 ± 0.3
Alb/globulin ratio	2.8 ± 0.4	2.4 ± 0.2	2.4 ± 0.2	3.9 ± 0.3	4.0 ± 0.7
Glucose, mg/dL	191 ± 19	162 ± 30	142 ± 17	160 ± 6	137 ± 15
Cholesterol, mg/dL	84 ± 3	86 ± 4	99 ± 6	82 ± 8	73 ± 8
Bilirubin, mg/dL	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0*	0.7 ± 0.1*
BUN, mg/dL	17 ± 1	17 ± 1	15 ± 2	20 ± 3	23 ± 2
Creatinine, mg/dL	1.6 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	1.6 ± 0.2
BUN/creatinine ratio	11 ± 1	13 ± 1	13 ± 2	12 ± 1	14 ± 1
Calcium, mg/dL	11.7 ± 0.5	11.1 ± 0.3	11.3 ± 0.2	11.9 ± 0.3	11.8 ± 0.5
Phosphorus, mg/dL	11.4 ± 0.9	10.9 ± 0.5	11.4 ± 0.9	12.7 ± 0.1	9.9 ± 0.4
Chloride, mEq/L	100 ± 1	99 ± 1	100 ± 1	101 ± 1	101 ± 2

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control at ($p \leq 0.05$).

and long-term testing, as well as pharmacokinetic and pharmacodynamic studies, are indicated.

Acute toxicity data for DBAN and DCAN are common et al. (*12*), Bull (*13*), and Meier et al. (*14*) suggest that the HAN may be biologically reactive, directly or by conversion to toxic products. Thorough short-term

Table 20. Organ weights and ratios of male CD rats exposed to dichloroacetonitrile by gavage for 14 days.[a]

Organ	Vehicle (corn oil)	DCAN dose 12 mg/kg	DCAN dose 23 mg/kg	DCAN dose 45 mg/kg	DCAN dose 90 mg/kg
Body weight, g	157 ± 5	170 ± 7	147 ± 5	137 ± 5	115 ± 4
Brain, mg	1680 ± 90	1630 ± 120	1660 ± 30	1560 ± 40	1520 ± 50*
% body weight	1.06 ± 0.03	0.99 ± 0.09	1.14 ± 0.04	1.15 ± 0.04	1.34 ± 0.06*
Liver, mg	8150 ± 310	9960 ± 580	9710 ± 640	10110 ± 520*	8630 ± 480
% body weight	5.19 ± 0.10	5.85 ± 0.16*	6.57 ± 0.30*	7.37 ± 0.18*	7.50 ± 0.26*
% brain weight	494 ± 23	687 ± 122	586 ± 39	650 ± 33*	576 ± 44
Spleen, mg	540 ± 40	620 ± 70	460 ± 30	470 ± 50	350 ± 30*
% body weight	0.34 ± 0.02	0.36 ± 0.04	0.31 ± 0.01	0.34 ± 0.03	0.30 ± 0.02
% brain weight	32.6 ± 2.4	40.9 ± 6.5	27.6 ± 1.5	30.2 ± 3.2	23.1 ± 1.8*
Lungs, mg	1270 ± 70	1370 ± 110	1260 ± 80	1170 ± 70	1020 ± 90
% body weight	0.81 ± 0.04	0.82 ± 0.07	0.85 ± 0.05	0.86 ± 0.05	0.89 ± 0.07
% brain weight	76.9 ± 4.6	88.1 ± 9.0	75.0 ± 4.0	75.0 ± 3.8	67.6 ± 5.6
Thymus, mg	430 ± 40	550 ± 50	380 ± 20*	330 ± 20*	270 ± 20*
% body weight	0.27 ± 0.02	0.32 ± 0.03	0.26 ± 0.02	0.24 ± 0.02	0.24 ± 0.02*
% brain weight	25.4 ± 1.7	37.1 ± 6.4*	23.1 ± 1.4	21.3 ± 1.2	18.2 ± 1.5*
Kidneys, mg	1530 ± 50	1850 ± 80*	1520 ± 50	1480 ± 60	1380 ± 70
% body weight	0.98 ± 0.03	1.10 ± 0.04*	1.03 ± 0.02	1.08 ± 0.02	1.20 ± 0.04*
% brain weight	93.3 ± 5.1	125.3 ± 20.0*	91.2 ± 2.3	94.6 ± 3.5	91.5 ± 4.3
Testes, mg	1540 ± 130	1740 ± 150	1540 ± 120	1260 ± 130	1250 ± 140
% body weight	0.97 ± 0.07	1.00 ± 0.06	1.03 ± 0.06	0.90 ± 0.09	1.07 ± 0.11
% brain weight	91.3 ± 5.4	126.8 ± 32.8	91.0 ± 6.5	80.3 ± 9.0	80.4 ± 9.3

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control at ($p \leq 0.05$).

Table 21. Organ weights and ratios of female CD rats exposed to dichloroacetonitrile by gavage for 14 days.[a]

Organ	Vehicle (corn oil)	DCAN dose 12 mg/kg	DCAN dose 23 mg/kg	DCAN dose 45 mg/kg	DCAN dose 90 mg/kg
Body weight, g	148 ± 6	147 ± 6	143 ± 4	146 ± 5	113 ± 5
Brain, mg	1610 ± 40	1520 ± 80	1470 ± 60	1610 ± 50	1450 ± 40
% body weight	1.09 ± 0.04	1.04 ± 0.04	1.03 ± 0.03	1.11 ± 0.07	1.30 ± 0.06*
Liver, mg	8060 ± 530	8490 ± 350	10560 ± 500*	11140 ± 570*	7780 ± 920
% body weight	5.40 ± 0.20	5.81 ± 0.15	7.37 ± 0.27*	7.59 ± 0.23*	7.07 ± 0.82*
% brain weight	502.9 ± 33.9	565.7 ± 22.6	721.9 ± 31.8*	704.8 ± 53.8*	544.7 ± 67.7
Spleen, mg	440 ± 30	430 ± 40	400 ± 20	430 ± 20	290 ± 20*
% body weight	0.30 ± 0.02	0.30 ± 0.03	0.28 ± 0.02	0.29 ± 0.02	0.26 ± 0.01
% brain weight	27.8 ± 2.3	29.0 ± 2.7	27.3 ± 1.3	26.8 ± 1.5	20.2 ± 1.0*
Lungs, mg	1350 ± 90	1170 ± 90	1130 ± 70	1230 ± 100	960 ± 70*
% body weight	0.91 ± 0.06	0.80 ± 0.05	0.79 ± 0.05	0.84 ± 0.06	0.86 ± 0.07
% brain weight	85.0 ± 7.4	77.6 ± 6.1	77.3 ± 4.6	77.1 ± 6.9	65.6 ± 4.2
Thymus, mg	480 ± 30	420 ± 40	380 ± 30	420 ± 20	320 ± 20*
% body weight	0.33 ± 0.02	0.29 ± 0.03	0.27 ± 0.02	0.29 ± 0.02	0.29 ± 0.02
% brain weight	30.2 ± 1.8	28.6 ± 3.1	26.0 ± 1.7	26.1 ± 1.6	22.2 ± 1.8*
Kidneys, mg	1520 ± 50	1420 ± 60	1470 ± 40	1510 ± 60	1280 ± 50*
% body weight	1.03 ± 0.03	0.97 ± 0.03	1.03 ± 0.03	1.03 ± 0.02	1.14 ± 0.04
% brain weight	95.2 ± 3.8	95.1 ± 4.7	101.7 ± 5.5	95.2 ± 5.7	88.0 ± 2.7
Ovaries, mg	110 ± 10	100 ± 10	100 ± 10	100 ± 10	80 ± 10*
% body weight	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
% brain weight	6.9 ± 0.0	7.0 ± 0.0	6.9 ± 0.0	6.2 ± 0.0	5.3 ± 0.0

[a] All data expressed as mean ± SEM.
*Significantly different from vehicle control ($p \leq 0.05$)..

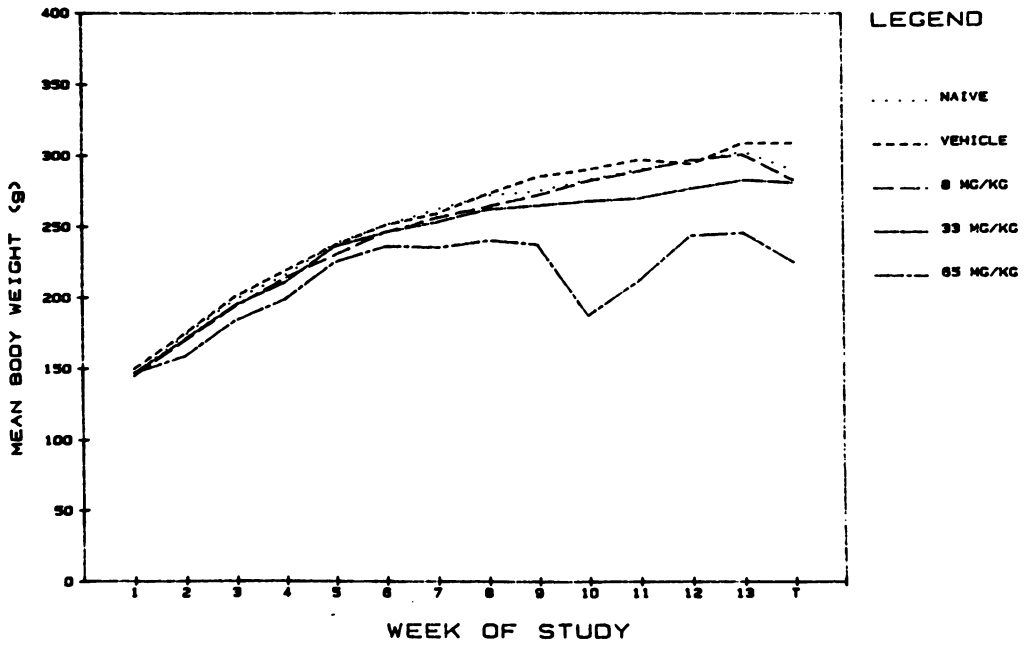


FIGURE 7. Weekly body weights of male CD rats exposed to dichloroacetonitrile for 13 weeks.

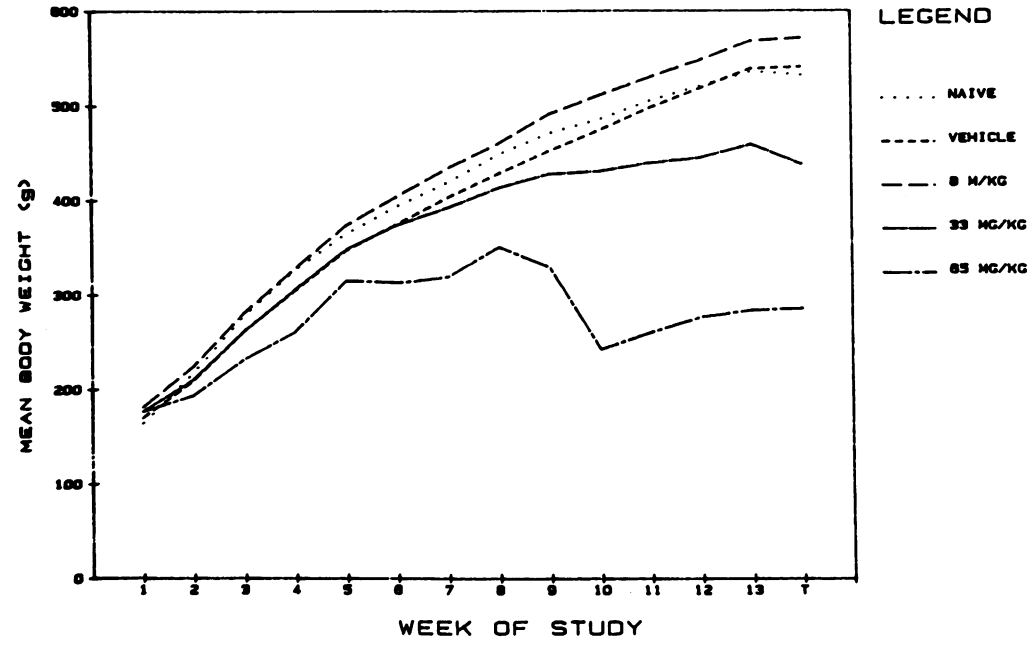


FIGURE 8. Weekly body weights of female CD rats exposed to dichloroacetonitrile for 13 weeks.

Table 22. Serum chemistry mean values for CD male rats exposed to dichloroacetonitrile at day 30 and 31 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
SGPT, IU/L	47 ± 7	47 ± 4	45 ± 1	55 ± 4	64 ± 3*
SGOT, IU/L	195 ± 32	219 ± 35	187 ± 24	178 ± 22	177 ± 13
ALP, IU/L	268 ± 27	273 ± 26	370 ± 34	453 ± 33*	541 ± 41*
5'-Nucleotidase, IU/L	13 ± 1	15 ± 1	16 ± 1	16 ± 1	19 ± 1
Protein, g/dL	5.9 ± 0.1*	7.0 ± 0.2	6.8 ± 0.2	6.3 ± 0.2*	6.1 ± 0.1*
Albumin, g/dL	4.8 ± 0.1	4.7 ± 0.1	5.1 ± 0.1*	4.8 ± 0.1	4.9 ± 0.1
Globulin, g/dL	1.1 ± 0.1*	2.3 ± 0.3	1.7 ± 0.2	1.6 ± 0.2*	1.2 ± 0.1*
Alb/globulin ratio	4.7 ± 0.3	2.3 ± 0.3	3.6 ± 0.6	3.4 ± 0.3	4.6 ± 0.4
Glucose, mg/dL	163 ± 20	141 ± 14	147 ± 20	148 ± 1	134 ± 6
Cholesterol, mg/dL	65 ± 2*	77 ± 5	84 ± 3	59 ± 3*	58 ± 2*
Bilirubin, mg/dL	0.4 ± 0.0*	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
BUN, mg/dL	19 ± 1	17 ± 1	18 ± 0	17 ± 1	14 ± 1*
Creatinine, mg/dL	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0	1.2 ± 0.0*	1.4 ± 0.1*
BUN/creatinine ratio	19 ± 1	20 ± 2	20 ± 1	13 ± 1*	9 ± 1*
Calcium, mg/dL	10.5 ± 0.2	11.6 ± 0.4	11.8 ± 0.4	11.1 ± 0.4	11.3 ± 0.2
Phosphorus, mg/dL	8.2 ± 0.2*	9.2 ± 0.4	8.6 ± 0.1	8.7 ± 0.2	7.7 ± 0.3*
Chloride, mEq/L	99 ± 1	100 ± 1	101 ± 1	99 ± 1	100 ± 1

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control at ($p \leq 0.05$).

Table 23. Serum chemistry mean values for CD male rats exposed to dichloroacetonitrile at day 59 and 60 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
SGPT, IU/L	34 ± 2	34 ± 3	36 ± 2	50 ± 2*	47 ± 1*
SGOT, IU/L	170 ± 19	176 ± 16	176 ± 11	200 ± 12	225 ± 40
ALP, IU/L	268 ± 38	298 ± 31	431 ± 40	688 ± 43*	686 ± 90*
5'-Nucleotidase, IU/L	17 ± 1	18 ± 2	18 ± 2	18 ± 1	15 ± 1
Protein, g/dL	6.2 ± 0.1*	7.6 ± 0.3	6.9 ± 0.2*	6.4 ± 0.8	5.8 ± 0.2*
Albumin, g/dL	5.3 ± 0.1*	4.8 ± 0.1	5.4 ± 0.1*	5.2 ± 0.1*	4.7 ± 0.0
Globulin, g/dL	0.9 ± 0.1*	2.8 ± 0.4	1.5 ± 0.2*	1.2 ± 0.1*	1.1 ± 0.2*
Alb/globulin ratio	6.5 ± 0.9*	2.2 ± 0.5	4.4 ± 1.1	4.6 ± 0.4	4.9 ± 0.7
Glucose, mg/dL	138 ± 5	133 ± 2	125 ± 7	133 ± 8	162 ± 19
Cholesterol, mg/dL	60 ± 2*	92 ± 11	89 ± 7	71 ± 5	56 ± 5*
Bilirubin, mg/dL	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.7 ± 0.1*
BUN, mg/dL	18 ± 1*	15 ± 1	15 ± 1	16 ± 1	12 ± 1
Creatinine, mg/dL	1.1 ± 0.0	1.0 ± 0.0	1.2 ± 0.1	1.3 ± 0.1*	1.2 ± 0.1
BUN/creatinine ratio	16 ± 1	16 ± 1	13 ± 1	12 ± 1	10 ± 0.0
Calcium, mg/dL	10.1 ± 0.2	10.6 ± 0.4	10.8 ± 0.2	10.1 ± 0.2	9.5 ± 0.3
Phosphorus, mg/dL	6.6 ± 0.3	6.9 ± 0.4	6.8 ± 0.3	6.8 ± 0.4	6.1 ± 0.2
Chloride, mEq/L	106 ± 1	104 ± 1	104 ± 1	106 ± 1	108 ± 1

[a]All data expressed as mean ± SEM.
*Significantly different from vehicle control at $p \leq 0.05$.

pared with data for acetonitrile and acrylonitrile in Table 30. The toxicity of the DHAN appears to be intermediate between that of acetonitrile and that of acrylonitrile. The rat and mouse appear to be equally sensitive to the acute effects of DCAN; however, the male rat appears to be more sensitive to DBAN than the female rat.

The data obtained following 14- and 90-day repeated oral administration of DBAN or DCAN to rats revealed a dose-dependent decrease in weight gain. Weight gain is a sensitive indicator of the general health status of the animal, and the decrease suggests that the body is responding to the toxic effects of DBAN and DCAN. The nature of this reaction is not evident, although it is unlikely that it is caused by chronic cyanide intoxication. The biochemical data failed to identify specific target organs. Organ weight and ratio data suggest that the thymus, liver, spleen, and gonads may be possible target organs. No consistent, compound-related and dose-dependent adverse effects were noted except the effect on body weight. The NOAEL for DBAN was determined to be 23 mg/kg/day; for DCAN, the NOAEL was identified as 8 mg/kg/day.

Insight into possible mechanisms of toxicity and significance of the data awaits further studies. For example, gavage administration using a corn oil vehicle

Table 24. Serum chemistry mean values for CD male rats exposed to dichloroacetonitrile at termination of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
SGPT, IU/L	38 ± 4	53 ± 1	45 ± 2	116 ± 60	53 ± 4
SGOT, IU/L	184 ± 17	195 ± 28	140 ± 13	265 ± 96	163 ± 18
ALP, IU/L	232 ± 28	222 ± 20	320 ± 31	471 ± 46*	603 ± 79*
5'Nucleotidase, IU/L	16 ± 1	19 ± 2	14 ± 1	22 ± 3	21 ± 1
Protein, g/dL	6.8 ± 0.1*	7.5 ± 0.1	7.2 ± 0.1	7.0 ± 0.1*	6.5 ± 0.1
Albumin, g/dL	5.8 ± 0.1	5.6 ± 0.1	6.0 ± 0.1*	5.6 ± 0.1*	5.4 ± 0.1
Globulin, g/dL	1.0 ± 0.1	1.9 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.0 ± 0.1
Alb/globulin ratio	6.1 ± 0.4	3.1 ± 0.4	5.3 ± 0.4	4.0 ± 0.4	5.0 ± 1
Glucose, mg/dL	140 ± 5	145 ± 4	143 ± 3	147 ± 4	109 ± 4*
Cholesterol, mg/dL	68 ± 3	81 ± 6	92 ± 6	71 ± 5	55 ± 4*
Bilirubin, mg/dL	0.4 ± 0.0*	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.1
BUN, mg/dL	20 ± 0.6*	14 ± 1	13 ± 1	14 ± 1	16 ± 2
Creatinine, mg/dL	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
BUN/creatinine ratio	17.9 ± 1	13.8 ± 1	11.7 ± 0.7	11.4 ± 0.7	13.2 ± 1
Calcium, mg/dL	9.3 ± 0.1*	10.1 ± 0.2	9.4 ± 0.1*	9.6 ± 0.2	9.2 ± 0.3*
Phosphorus, mg/dL	5.5 ± 0.2	5.7 ± 0.3	5.1 ± 0.2	6.1 ± 0.4	5.9 ± 0.3
Chloride, mEq/L	105 ± 1	105 ± 1	103 ± 1	103 ± 1	107 ± 1

[a] All data expressed as mean ± SEM.
* Significantly different from vehicle control at $p \leq 0.05$.

Table 25. Serum chemistry values for CD female rats exposed to dichloroacetonitrile at day 30 and 31 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
SGPT, IU/L	43 ± 4	38 ± 3	39 ± 8	42 ± 3	44 ± 2
SGOT, IU/L	178 ± 23	171 ± 12	175 ± 11	138 ± 12	161 ± 22
ALP, IU/L	205 ± 16	223 ± 16	202 ± 21	210 ± 12	269 ± 17
5'-Nucleotidase, IU/L	30 ± 3	34 ± 3	24 ± 2*	18 ± 1*	21 ± 1*
Protein, g/dL	6.3 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.7 ± 0.2	6.0 ± 0.2*
Albumin, g/dL	5.2 ± 0.1	5.6 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	4.9 ± 0.2*
Globulin, g/dL	1.1 ± 0.1	1.0 ± 0.1	1.6 ± 0.1*	1.2 ± 0.1	1.1 ± 0.2
Alb/globulin ratio	5.3 ± 0.6	6.2 ± 0.8	3.5 ± 0.2*	4.7 ± 0.4	5.3 ± 0.7
Glucose, mg/dL	147 ± 6	145 ± 3	138 ± 4	127 ± 6	143 ± 8
Cholesterol, mg/dL	80 ± 4	86 ± 3	73 ± 5	76 ± 4	56 ± 3*
Bilirubin, mg/dL	0.4 ± 0.0*	0.6 ± 0.0	0.5 ± 0.0*	0.5 ± 0.0*	0.4 ± 0.0*
BUN, mg/dL	19 ± 1	17 ± 1	18 ± 1	21 ± 1	20 ± 1
Creatinine, mg/dL	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	1.3 ± 0.0	1.4 ± 0.1*
BUN/creatinine ratio	19 ± 1	18 ± 1	20 ± 2	16 ± 1	14 ± 1*
Calcium, mg/dL	10.6 ± 0.2	10.8 ± 0.3	10.8 ± 0.3	11.2 ± 0.3	10.5 ± 0.2
Phosphorus, mg/dL	7.9 ± 0.2*	6.8 ± 0.3	7.4 ± 0.3	6.9 ± 0.3	7.0 ± 0.2
Chloride, mEq/L	100 ± 2	101 ± 1	97 ± 1	99 ± 2	99 ± 1

[a] All data expressed as mean ± SEM.
* Significantly different from vehicle control at $p \leq 0.05$.

Table 26. Serum chemistry mean values for CD female rats exposed to dichloroacetonitrile at day 59 and 60 of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
SGPT, IU/L	36 ± 5	33 ± 2	40 ± 5	51 ± 12	35 ± 4
SGOT, IU/L	143 ± 10	143 ± 12	163 ± 24	198 ± 36	225 ± 97
ALP, IU/L	188 ± 20	207 ± 21	260 ± 28	295 ± 21	557 ± 79*
5'-Nucleotidase, IU/L	28 ± 3	32 ± 2	19 ± 2*	18 ± 2*	16 ± 3*
Protein, g/dL	6.6 ± 0.1*	7.2 ± 0.2	6.9 ± 0.1	6.8 ± 0.1	6.0 ± 0.2*
Albumin, g/dL	5.7 ± 0.1	5.6 ± 0.1	6.0 ± 0.1*	5.9 ± 0.1	5.3 ± 0.1
Globulin, g/dL	0.9 ± 0.1*	1.5 ± 0.2	0.9 ± 0.1*	0.8 ± 0.1*	0.6 ± 0.2*
Alb/globulin ratio	7.3 ± 0.1	4.3 ± 0.6	7.4 ± 0.8	8.0 ± 1.0*	9.4 ± 2.0*
Glucose, mg/dL	144 ± 4	137 ± 3	140 ± 6	143 ± 13	176 ± 16*
Cholesterol, mg/dL	81 ± 4	78 ± 4	83 ± 6	73 ± 3	50 ± 1*
Bilirubin, mg/dL	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.3 ± 0.0*	0.6 ± 0.0
BUN, mg/dL	20 ± 0*	16 ± 0	17 ± 1	19 ± 1*	13 ± 1
Creatinine, mg/dL	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.4 ± 0.2
BUN/creatinine ratio	17 ± 1	14 ± 1	15 ± 1	14 ± 1	10 ± 1
Calcium, mg/dL	10.3 ± 0.1	10.5 ± 0.3	10.3 ± 0.1	10.6 ± 0.1	10.2 ± 0.3
Phosphorus, mg/dL	5.7 ± 0.1	5.4 ± 0.3	5.1 ± 0.2	5.1 ± 0.3	4.4 ± 0.5
Chloride, mEq/L	105 ± 1	107 ± 1	106 ± 1	105 ± 1	107 ± 3

[a] All data expressed as mean ± SEM.
* Significantly different from vehicle control at $p \leq 0.05$.

Table 27. Serum chemistry mean values for CD female rats exposed to dichloroacetonitrile at termination of subchronic study.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
SGPT, IU/L	51 ± 7	51 ± 3	32 ± 2*	27 ± 2*	35 ± 3*
SGOT, IU/L	198 ± 29*	120 ± 14	127 ± 15	124 ± 14	126 ± 16
ALP, IU/L	146 ± 20	228 ± 29	226 ± 25	286 ± 30	499 ± 46*
5-Nucleotidase, IU/L	28 ± 2	30 ± 2	19 ± 1*	16 ± 1*	17 ± 1*
Protein, g/dL	6.9 ± 0.1	7.1 ± 0.1	7.3 ± 0.1	7.1 ± 0.1	6.6 ± 0.1*
Albumin, g/dL	6.1 ± 0.1	6.2 ± 0.2	6.0 ± 0.1	6.0 ± 0.1	5.5 ± 0.1*
Globulin, g/dL	0.8 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Alb/globulin ratio	8.4 ± 0.7	7.0 ± 1	5.1 ± 0.4	6.0 ± 0.4	5.0 ± 1
Glucose, mg/dL	144 ± 8	150 ± 6	127 ± 5*	140 ± 5	120 ± 6*
Cholesterol, mg/dL	92 ± 5*	73 ± 3	96 ± 6*	80 ± 7	54 ± 4*
Bilirubin, mg/dL	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
BUN, mg/dL	19 ± 1*	15 ± 1	16 ± 1	15 ± 1	16 ± 1
Creatinine, mg/dL	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	1.4 ± 0.1*
BUN/creatinine ratio	16 ± 1	14 ± 1	13 ± 1	12 ± 1	12 ± 1
Calcium, mg/dL	9.6 ± 0.1	9.7 ± 0.1	10.1 ± 0.2	9.8 ± 0.2	9.8 ± 0.2
Phosphorus, mg/dL	5.4 ± 0.2	5.0 ± 0.2	5.1 ± 0.2	5.4 ± 0.2	5.0 ± 0.2
Chloride, mEq/L	105 ± 1	107 ± 1	106 ± 2	105 ± 1	106 ± 1

[a] All data expressed as mean ± SEM.
* Significantly different from vehicle control at $p \leq 0.05$.

Table 28. Organ weights and ratios of male CD rats exposed to dichloroacetonitrile by gavage for 90 days.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
Body weight, g	532.5 ± 11.1	541.4 ± 13.1	572.1 ± 14.5	438.0 ± 16.6*	285.6 ± 20.6*
Brain, mg	1820 ± 60	1860 ± 60	1840 ± 70	1860 ± 60	1770 ± 60
% body weight	0.34 ± 0.01	0.35 ± 0.01	0.33 ± 0.02	0.44 ± 0.02*	0.63 ± 0.03*
Liver, mg	19300 ± 750	21430 ± 730	25830 ± 1040	27260 ± 1060	17910 ± 1600
% body weight	3.6 ± 0.50	4.0 ± 0.10	4.5 ± 0.13	6.4 ± 0.43*	6.4 ± 0.92*
% brain weight	1080 ± 51	1170 ± 47	1430 ± 86*	1500 ± 62*	1022.5 ± 128
Spleen, mg	780 ± 40	720 ± 30	730 ± 30	630 ± 30	500 ± 60*
% body weight	0.15 ± 0.03	0.13 ± 0.03	0.13 ± 0.02	0.15 ± 0.04	0.18 ± 0.03*
% brain weight	44 ± 3.0	40 ± 2.0	41 ± 3.0	35 ± 2.0	29.0 ± 4.0
Lungs, mg	2700 ± 120	2980 ± 130	2770 ± 130	2630 ± 100	1830 ± 60*
% body weight	0.51 ± 0.02	0.56 ± 0.02	0.49 ± 0.02*	0.61 ± 0.02	0.65 ± 0.04
% brain weight	150.0 ± 6.0	160.0 ± 8.0	150.0 ± 10.0	140.0 ± 6.0	103.0 ± 1.0*
Thymus, mg	580 ± 30	600 ± 30	790 ± 40*	620 ± 30	310 ± 30*
% body weight	0.11 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.01*	0.11 ± 0.02
% brain weight	33.0 ± 2.0	33.0 ± 2.0	44.0 ± 3.0*	34.0 ± 2.0	18.0 ± 3.0*
Kidneys, mg	3840 ± 80	3710 ± 70	3880 ± 120	3450 ± 120	2770 ± 190*
% body weight	0.73 ± 0.02	0.69 ± 0.01	0.68 ± 0.02	0.81 ± 0.04*	1.0 ± 0.12*
% brain weight	210.0 ± 8.0	200.0 ± 8.0	220.0 ± 13.0	190.0 ± 8.0	160.0 ± 15.0
Testes, mg	3490 ± 80	3420 ± 80	3660 ± 60	3400 ± 70	2910 ± 150*
% body weight	0.66 ± 0.02	0.63 ± 0.02	0.65 ± 0.02	0.80 ± 0.04*	1.0 ± 0.12*
% brain weight	200.0 ± 8.0	190.0 ± 8.0	200.0 ± 10.0	190.0 ± 6.0	170.0 ± 14.0

[a] All data expressed as mean ± SEM.
* Significantly different from vehicle control at $p \leq 0.05$.

does not simulate human exposure conditions. This issue is currently being addressed by the National Toxicology Program. Limited water solubility and lack of acceptance by rats in taste aversion studies necessitates the use of a solvent to assess effects at high doses. Limit studies (solubility limit in water) should be considered. The histopathological evaluation of major organs (tissues) from exposed animals should provide some infor-

Table 29. Organ weights and ratios of female CD rats exposed to dichloroacetonitrile by gavage for 90 days.[a]

Parameter	Naive	Vehicle (corn oil)	DCAN dose 8 mg/kg	DCAN dose 33 mg/kg	DCAN dose 65 mg/kg
Body weight, g	290.0 ± 7.3	309.0 ± 6.91	282.5 ± 17.4	280.6 ± 10.7	225.5 ± 8.9*
Brain, mg	1790 ± 40	1680 ± 70	1690 ± 70	1580 ± 60	1460 ± 80
% body weight	0.63 ± 0.02	0.55 ± 0.02	0.57 ± 0.02	0.58 ± 0.03	0.66 ± 0.05
Liver, mg	11000 ± 540	12100 ± 420	14130 ± 870	16830 ± 580*	13880 ± 705
% body weight	3.8 ± 0.11	4.0 ± 0.14	4.7 ± 0.29*	6.1 ± 0.22*	6.1 ± 0.15*
% brain weight	615.0 ± 27.8	737.2 ± 37.5	1430 ± 86*	1092.5 ± 55.9*	993.5 ± 98.4*
Spleen, mg	650 ± 120	540 ± 20	560 ± 30	500 ± 20	450 ± 20
% body weight	0.22 ± 0.04	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
% brain weight	36.6 ± 6.5	33.1 ± 1.9	34.4 ± 2.6	32.6 ± 2.2	31.9 ± 2.6
Lungs, mg	1970 ± 90	2040 ± 100	2200 ± 150	2220 ± 160	1830 ± 100
% body weight	0.68 ± 0.03	0.67 ± 0.03	0.74 ± 0.05	0.79 ± 0.03*	0.82 ± 0.05*
% brain weight	109.8 ± 3.7	1226 ± 6.9	134.7 ± 13.0	148.3 ± 16.5	130.4 ± 12.8
Thymus, mg	430 ± 10	500 ± 30	540 ± 30	420 ± 20*	375 ± 20*
% body weight	0.15 ± 0.004	0.16 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
% brain weight	24.3 ± 1.0	30.6 ± 1.8	33.0 ± 2.4	27.5 ± 2.3	26.5 ± 1.9
Kidneys, mg	2230 ± 70	2230 ± 60	2380 ± 60	2200 ± 80	2250 ± 120
% body weight	0.77 ± 0.02	0.72 ± 0.01	0.80 ± 0.02	0.80 ± 0.03	1.00 ± 0.02*
% brain weight	125.2 ± 3.8	136.7 ± 6.9	145.4 ± 8.4	143.5 ± 7.7	163.6 ± 17.3
Ovaries, mg	140 ± 10	160 ± 10	140 ± 10	140 ± 10	100 ± 20*
% body weight	0.05 ± 0.003	0.05 ± 0.004	0.05 ± 0.002	0.05 ± 0.003	0.05 ± 0.01
% brain weight	8.2 ± 0.6	9.9 ± 1.0	8.1 ± 0.4	9.5 ± 0.7	7.1 ± 1.4

[a] All data expressed as mean ± SEM.
* Significantly different from vehicle control at $p \leq 0.05$.

Table 30. A comparison of acute toxicity data of a series of nitriles.

Study	Acrylonitrile	Acetonitrile	Dibromoacetonitrile	Dichloroacetonitrile
Oral LD_{50}, mg/kg				
Rat				
M	93	3,800	245 (210–286)	389 (298–387)
F			361 (320–410)	330 (300–500)
Mouse				
M	27		289 (253–324)	270 (241–303)
F			303 (269–342)	279 (263–296)
Inhalation LC_{50}				
Rat				
M		7,551 ppm/8H		
F		12,435 ppm/8H		
Dermal LD_{50}				
Rabbit	0.25 ml/kg	1,250 mg/kg		
Mutagenicity				
S. typhimurium	Positive		Negative (?)	Positive

mation on effects at the cellular level. The pharmacokinetic and pharmacodynamic studies on HAN in corn oil, in water, or in other solvents, administered by gavage and in drinking water, should be considered.

Estimates of human exposure to HAN in drinking water and from other sources including occupational exposure are lacking. Lifetime toxicity/oncogenicity studies are indicated since the HAN are reported to be mutagenic and probably carcinogenic. The definitive answer resides in chronic toxicity studies in intact animals.

These studies were supported by Cooperative Agreement No. 808861 from the Environmental Protection Agency.

This paper has been reviewed in accordance with the U.S. Environmental Protection Agency's peer and administrative review policies and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

1. Rook, J. J. Formation of haloforms during chlorination of natural waters. Water Treat. Exam. 23: 234–243 (1974).
2. Rook, J. J. Chlorination reactions of fulvic acids in natural waters. Environ. Sci. Technol. 11: 478–482 (1977).
3. Bellar, T. A., Lichtenberg, J. J., and Kroner, R. C. Occurrence of organohalides in chlorinated drinking waters. J. Am. Water Works Assoc. 66: 703–706 (1974).
4. Symons, J. M., Bellar, T. A., Carswell, J., DeMarco, J., Kropp, K. L., Robeck, G. C., Seegar, D. R., Slocum, C. J., Smith, B. L., and Stevens, A. A. National organics reconnaissance survey for halogenated organics. J. Am. Water Works Assoc. 67: 634–647 (1975).
5. McKinney, J. D., Mauer, R. R., Haas, J. R., and Thomas, R. O. Possible factors in drinking water of laboratory animals causing reproductive failure. In: Identification and Analysis of Organic Pollutants in Water (L. H. Keith, Ed.), Ann Arbor Science Publishers, Ann Arbor, MI, 1976, pp. 417–432.
6. Stevens, A. A., and Seeger, D. R. Formation of non-polar organochloro compounds as byproducts of chlorination. In: NATO/Comm. Challenges Mod. Soc. 1979, pp. 145–160.
7. Trehy, M. L., and Bieber, T. I. Detection, identification and quantitative analysis of dihaloacetonitriles in chlorinated natural waters. In: Advances in the Identification and Analysis of Organic Pollutants in Water, Vol. 2 (L. H. Keith, Ed.), Ann Arbor Science Publishers, Ann Arbor, MI, 1981, pp. 941–975.
8. Oliver, B. G. Dihaloacetonitriles in drinking water: algae and fulvic acid as precursors. Environ. Sci. Technol. 17: 80–83 (1983).
9. Mink, F. L., Coleman, W. E., Munch, J. W., Kaylor, W. H., and Ringhand, H. P. *In vivo* formation of halogenated reaction products following peroral sodium hypochlorite. Bull. Environ. Contam. Toxicol. 30: 394–399 (1983).
10. Cotton, R. T., and Walkden, H. H. The role of sorbtion in the fumigation of stored grain and cereal products. J. Kansas Entomol. Soc. 17: 98–103 (1944).
11. Matt, J. U.S. Patent 3,608,084 (1968).
12. Simmon, V. F., Kauhanen, K., and Tardiff, R. G. Mutagenic activity of chemicals identified in drinking water. In: Progress in Genetic Toxicology (D. Scott, B. A. Bridges and F. H. Sobels, Eds.), Elsevier/North-Holland Biomedical Press, 1977, pp. 249–258.
13. Bull, R. J. Experimental methods for evaluating the health risks associated with organic chemicals in drinking water. Toxicol. Environ. Chem. 6: 1–17 (1982).
14. Meier, J. R., Baker, K. L., Bull, R. J., and Robinson, M. R. Mutagenicity and tumor initiating activity of halogenated acetonitriles. Environ. Mutagen. 5: 447–448 (1983).
15. Kraybill, H. F. Evaluation of public health aspects of carcinogenic/mutagenic biorefractories in drinking water. Preventive Med. 9: 212–218 (1980).
16. Kraybill, H. F. Assessment of human exposure and health risk to environmental contaminants in the atmosphere and water with special reference to cancer. J. Environ. Sci. Health CI(2): 175–232 (1983).
17. U.S. Food and Drug Administration. Toxicological Principles for the Safety Assessment of Direct Food Additives. 1982.
18. Organization for Economic Cooperation and Development. Guidelines for Testing of Chemicals. 1981.
19. Litchfield, J. T., Jr., and Wilcoxon, F. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exptl. Therap. 96: 99–113 (1949).
20. Pereira, M. A., Lin, E. H. C., and Mattox, J. K. Haloacetonitrile excretion as thiocyanate and inhibition of dimethylnitrosamine demethylase: a proposed metabolic scheme. J. Toxicol. Environ. Health 13: 633–641 (1984).
21. Daniel, F. B., Pereira, M. A., Lin, E. H. C., Hass, D. L., Mattox, J. K., and Schenck, K. Genotoxicity of haloacetonitriles. Toxicologist 3: 37 (1983).